

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
19 September 2002 (19.09.2002)

PCT

(10) International Publication Number  
**WO 02/072742 A1**

(51) International Patent Classification<sup>7</sup>: **C11B 1/10**,  
1/06, A23D 9/00, C12P 7/64, A23L 1/30, A23C 11/04,  
A23K 1/16, A61K 7/48

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(21) International Application Number: PCT/EP02/02333

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(22) International Filing Date: 7 March 2002 (07.03.2002)

(81) Designated States (*national*): AU, BR, CA, CZ, ID, IN,  
JP, MX, NO, NZ, PH, RU, SK, US, ZA.

(25) Filing Language: English

Declarations under Rule 4.17:

(26) Publication Language: English

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AU, BR, CA, CZ, ID, IN, JP, MX, NO, NZ, PH, RU, SK, ZA
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AU, BR, CA, CZ, ID, IN, JP, MX, NO, NZ, PH, RU, SK, ZA
- of inventorship (Rule 4.17(iv)) for US only

(30) Priority Data:  
01105960.7 9 March 2001 (09.03.2001) EP

Published:

(71) Applicant (for all designated States except US): SOCIETE DES PRODUITS NESTLE S.A. [CH/CH]; P.O. Box 353, CH-1800 Vevey (CH).

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(72) Inventors; and

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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(54) Title: OIL CONTAINING ONE OR MORE LONG-CHAIN POLYUNSATURATED FATTY ACIDS DERIVED FROM BIOMASS, PROCESS FOR PREPARING IT, FOODSTUFF, OR NUTRITIONAL, COSMETIC OR PHARMACEUTICAL COMPOSITION CONTAINING IT

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(57) Abstract: Stable oil containing LC-PUFAs in the form of triacylglycerols, in particular arachidonic acid (ARA), dihomogam-malinolenic acid (DHGLA), docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA). Such an oil may be prepared by bringing a carrier oil entering into the composition of a foodstuff, of a cosmetic or pharmaceutical product, into contact with a biomass obtained from the culture of a microorganism, in particular a fungus or a microalga containing the acids ARA, DHGLA, DHA or EPA.

OIL CONTAINING ONE OR MORE LONG-CHAIN POLYUNSATURATED FATTY ACIDS DERIVED FROM BIOMASS, PROCESS FOR PREPARING IT, FOODSTUFF, OR NUTRITIONAL, COSMETIC OR PHARMACEUTICAL COMPOSITION CONTAINING IT

5

Introduction

The present invention relates to the field of the preparation of an oil serving as an ingredient which is 10 a source of long-chain polyunsaturated essential fatty acids (LC-PUFA) in a foodstuff, in a nutritional supplement, in a cosmetic or pharmaceutical composition.

15 State of the art

An oil containing LC-PUFAs such as for example arachidonic acid (ARA), docosahexaenoic acid (DHA) or dihomogammalinolenic acid (DHGLA) may be obtained from 20 a biomass fermentation broth. To obtain the oil from the biomass, methods of extraction with organic solvent, for example hexane, or with supercritical fluid, have been used. Generally, the oil has been extracted from biomass by percolation of the dried 25 biomass with hexane.

Such a process of extraction with organic solvent(s) is described, for example, in WO9737032, in WO 9743362 or in the publication Journal of Dispersion Science and 30 Technology, 10, 561-579, 1989 "Biotechnological processes for the production of PUFAs".

This technique has various disadvantages:

- During the stages of extraction with hot 35 solvent or of distillation of the solvent, the LC-PUFAs may undergo degradation in contact with oxygen.

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- The complete removal of the solvent contained in the oil or in the residual biomass requires a heat treatment at high temperature.

5 - Moreover, the solvent, such as hexane, is capable of dissolving nontriacylglycerol constituents of the biomass which in fact constitute impurities.

10 The crude oil obtained after evaporation of the solvent should further undergo several refining stages comprising degumming, neutralization with alkali, decolorization, dewaxing and deodorization with the aim of at least partially removing the impurities. This means that the highly unsaturated oil is exposed to 15 conditions stimulating physicochemical reactions which affect its quality. For example, the decolorization agents create a system of conjugated double bonds and form degradation products by chemical reaction with the oxidized glycerides.

20 The aim of the present invention is to avoid the disadvantages of the prior art, by providing a stable oil containing one or more polyunsaturated fatty acids derived from biomass in the form of triacylglycerols in 25 the purified state and which has undergone minimum degradation.

#### Summary of the invention

30 The present invention relates to a stable oil containing LC-PUFAs in the form of triacylglycerols, in particular arachidonic acid (ARA), dihomogammalinolenic acid (DHGLA), docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA).

35 The invention also relates to a process for preparing such an oil by bringing a carrier oil entering into the composition of a foodstuff into contact with a biomass obtained from the culture of a microorganism, in

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particular a fungus or a microalga containing the acids ARA, DHGLA, DHA or EPA.

Preferably, the oil does not contain more than 10% by weight of polyunsaturated fatty acids. As a result, the oil is a lot less sensitive to oxidation during its production, which is not the case for the oils containing LC-PUFAs of the prior art.

10 According to a main aspect of the invention, it is a crucial qualitative advantage to have available a novel oil containing LC-PUFAs in the form of triacylglycerols.

15 According to another aspect, the invention relates to a foodstuff, a cosmetic or pharmaceutical product, a nutritional supplement or an animal feed containing the preceding oil.

20 According to yet another aspect, the invention relates to an animal feed, in particular for pets containing the biomass residue derived from the process.

Detailed description of the invention

25

The conversion is carried out by bringing the carrier oil into contact with a biomass containing LC-PUFAs. The oil is suitable for application in foodstuffs, in particular infant formulas or for use as a nutritional supplement. It may also be used in cosmetic or pharmaceutical products. Furthermore, the biomass residue obtained is also a product of the process which may be upgraded directly without subsequent treatment, for example as animal feed, in particular for pets.

35

The preparation of such an oil may take place by simply mixing the carrier oil with the dried biomass and subsequently separating the oil from the nonlipid solids by pressing.

In order to increase the yield of LC-PUFA obtained it is preferable to reduce the sizes of the particles of dry biomass in order to break the walls of the cells of microorganisms and to thereby increase the surface area of contact between the oil and the biomass. This may be appropriately carried out using various methods, for example:

- the biomass may be ground in the presence of the carrier oil;
- the biomass may be laminated before mixing it with the carrier oil;
- the biomass may be treated at high pressure in the presence of the carrier oil, and then the oil obtained may be separated from the biomass by pressing and final filtration;
- the biomass may be treated with enzymes capable of degrading the walls of the cells.

Because the carrier is an oil, the oil obtained after contact with the biomass has a minimum content of phospholipids, free fatty acids, pigments, polymers and other substances obtained or derived from the biomass which are not triacylglycerols. This means that the process according to the invention constitutes a selective method for preparing a stable purified oil containing LC-PUFAs. It is not necessary to purify the unsaturated oil containing the LC-PUFAs by the aggressive and cumbersome methods used prior to the invention such as the stages of degumming, neutralization, dewaxing and decolorization.

According to the invention the oil is solely subjected to a stage of deodorization, for example by steam distillation or molecular distillation at a relatively low temperature. The result is that the oil contains a particularly small quantity of trans fatty acids.

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The process does not use organic solvent and, since the operation is carried out under a nitrogen layer and in the presence of tocopherols which are naturally present or which are added to the carrier oil, the LC-PUFAs are  
5 protected from oxidative degradation during the whole process.

In addition to the quality of the oil obtained, another  
10 advantage of the process consists in the fact that the biomass residue is not contaminated with an organic solvent and may thus be directly upgraded, without subsequent treatment, for example in animal feed, in particular for pets.

15 The detailed description of the process which follows is targeted at the preparation of an oil containing ARA, taken by way of nonlimiting example. The working conditions for transferring other LC-PUFAs to a carrier oil from appropriate biomasses, for example for DHA or  
20 DHGLA, are very similar.

The oil is obtained by mixing the carrier oil with the dry biomass and separating the oil from the solid components by pressing. To increase the level of incorporation of ARA, it is desirable to break the  
25 microbial cells by high-pressure treatments, by enzymatic processes or to reduce the sizes of the dry particles of biomass by grinding or laminating.

30 The grinding step used may be one of many techniques known in the prior art, for example, the biomass may be laminated, preferably at low temperature, and then it may be mixed with the carrier oil. As a variant, the biomass may be ground in the presence of the carrier  
35 oil. In order to minimize as much as possible damage to the ARA, the grinding conditions should be gentle. In this regard, grinding the biomass in the presence of the carrier oil and under an inert atmosphere, for example under a nitrogen stream, is preferred.

Next, the oil containing the ARA is separated from the biomass by filtration or pressing, preferably at high pressure, and then a final filtration is carried out so  
5 as to remove the fine particles of biomass.

It was observed that the level of incorporation of the ARA increased when the size of the biomass particles decreased; it was > 90% when for example 90% of the  
10 particles had a size < 250 µm.

By way of example, it is possible to use a ball mill or a colloidal mill. The parameters to be considered are the duration of grinding, the size of the biomass  
15 particles, the grinding temperature, the ratio between the quantities of biomass and of carrier oil.

The duration of grinding has an influence on the size of the particles and the latter is also influenced by the grinding temperature. Consequently, in practice, it is preferable to indicate the size of the particles as a parameter determining the grinding stage. Thus, it is desirable that 90% of the particles have a size < 500 µm, preferably that 90% of the particles have a  
25 size < 300 µm and more preferably still that 90% of the particles have a size < 200 µm.

The grinding temperature is chosen at a value greater than the melting point of the carrier oil, and is  
30 preferably 20 to 80°C. To obtain an optimum level of incorporation, a brief grinding may be carried out at a high temperature or a prolonged grinding at a low temperature.

35 The weight ratio chosen between the biomass and the carrier oil determines the content of ARA of the final oil. Thus, for example, 30 parts of biomass are chosen per 70 parts of carrier oil in order to obtain at least 4.5% of ARA in the converted oil.

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The oil used as carrier may be any oil or mixture of oils which can be consumed as human food. An oil or a mixture entering into the composition of the product 5 which it is desired to enrich with PUFA is preferably used. There may be mentioned in particular for an infant formula high oleic acid sunflower oil (HOSFO), sunflower oil (SFO), soya bean oil, palm olein and a medium-chain triacylglycerol (MCT, containing 10 essentially triacylglycerols of saturated C<sub>8</sub>-C<sub>10</sub> fatty acids).

The next stage of the process consists in separating the spent biomass residue by any customary method such 15 as, for example, pressing, filtration or centrifugation. To this end, a press operating at high pressure is preferably used.

The oil obtained should be made free of fine insoluble 20 particles by fine filtration. This operation may be carried out, where appropriate, by exposing the oil to a mineral adsorbent as a filter aid, for example dicalite.

Finally, the filtered oil is deodorized in order to remove the volatile substances. This may be carried out 25 by any known method provided that moderate conditions are used in order to be gentle with the ARA. There may be mentioned, for example, steam distillation, preferably under vacuum, or molecular distillation.

The oil obtained may be used in food compositions for human consumption as it is or in the form of an emulsion such as, for example, oils, salad dressings or 35 mayonnaise. It may be a constituent of a dietetic milk for teenagers or adults, an infant formula for premature babies, full-term unweaned babies or a follow-on milk for small children.

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It may be incorporated into a nutritive or supplemental composition for oral consumption.

5 It may be incorporated into a pharmaceutical composition for oral, enteral or parenteral ingestion, or for topical, dermatological or ophthalmological application.

10 It may constitute an ingredient for a cosmetic, topical or oral composition.

Finally, it may constitute an ingredient for a pet food, for example a dry or moist food or a milk.

15 The biomass residue, after separation of the oil, may be advantageously used in animal feed, particularly for pets.

Examples

20

The examples below illustrate the invention. Therein, the parts and percentages are by weight, unless otherwise stated. The biomass:carrier oil ratio is 3:7.

25 Examples 1-6

In these examples, the process parameters and the quality of the oil obtained, before the final deodorization stage, compared with the starting carrier oil (reference 1) are studied. For the grinding, a ball mill is used. The results are summarized in Table 1 below:

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Table 1

Example	1	2	3	4	5	6	Reference 1
Grinding temperature (°C)	50	70	50	30	70	30	Carrier oil*
Grinding time (min)	3	5	3	5	1	1	-
Free fatty acids (%) IUPAC 2.201	0.13	0.13	0.14	0.12	0.17	0.15	0.14
Peroxide value (meq/kg) AOCS Cd 8b-90	4.0	4.1	3.5	3.5	4.0	3.4	2.7
Unsaponifiable components (g/kg) IUPAC 2.401	8.34	9.20	8.82	8.03	8.11	7.44	6.78
ARA (g ARA/100 g oil) IUPAC 2.304	4.4	4.87	4.25	4.46	3.81	3.27	0
Phosphorus (ppm) NI C12-1976-SSOG	2	1	3	2	2	4	6

\*The carrier oil is a high oleic acid sunflower oil,  
5 TRISUN™.

The theoretical value for incorporation of 100% of ARA is 5.3% with a biomass:carrier oil ratio of 3:7.

10 The purity of the crude oil may be qualified by the values below:

- Free fatty acids: 0.13-0.17% (TRISUN, carrier oil: 0.14%)
- Phosphorus: 1-4 ppm (part per million) (TRISUN: 6 ppm)

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- Unsaponifiable matter: 7.4-9.2 g/kg (TRISUN: 6.8 g/kg)

In conclusion:

- 5 - More than 60% of the ARA of the biomass is incorporated into the carrier oil.
- The phosphorus content is very low, a few ppm, approximately 100 x less than in the case of the crude oil extracted with hexane which was about 500 ppm.

Examples 7-10

15 In these examples, the process parameters and the quality of the oil obtained, after the final deodorization stage, during the use of various carrier oils, are studied. In these examples, the grinding of the biomass is carried out using a ball mill.

20 The characteristics of the oils obtained are compared with the crude oil obtained by extraction with hexane, without refining (reference 2) and compared with the oil obtained by direct pressing, and therefore with no carrier oil (reference 3).

The results are summarized in Table 2 below:

25

Table 2

Example	7	8	9	10	Reference 2	Reference 3
Carrier oil	HOSFO*	HOSFO*	MCT	Palm olein		
Grinding conditions: temperature (°C),	70	30	70	70		
Time (min)	5	10	5	5		
Free fatty acids (%)	0.04	0.03	0.04	0.04	0.56	0.11

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Example	7	8	9	10	Refer- ence 2	Refer- ence 3
Peroxide value (meq/kg)	2.0	2.3	2.3	1.3	11.5	4.8
Unsaponifi- fiable matter (g/kg)	8.02	7.05	3.53	5.40	22.89	17.59
Phosphorus (ppm)	3	4	4	3	508	17
ARA (g ARA/100g oil)	4.6	4.7	4.5	4.4	39.5	39.6

\*The carrier oil is a high oleic acid sunflower oil (HOSFO).

5 The results obtained allow the following conclusions:

- The grinding temperature and time are linked: a grinding of 10 min at 30°C gives the same level of incorporation of ARA as a grinding of 5 min at 70°C
- 10 - The level of incorporation depends only slightly on the type of carrier oil when the procedure is carried out at the same grinding temperature/time: high oleic acid sunflower oil (4.9% ARA at 70°C/5 min), MCT (5.0% ARA at 70°C/5 min) and palm olein (5.0% ARA at 70°C/5 min).
- 15 - A very small quantity of phosphorus is obtained compared to that obtained by extraction with hexane, which shows the purity of the final oil.

20 Examples 11-14

The examples below show the preparation of an oil containing ARA in the form of triacylglycerols by a process which is gentle with the quality of the ARA by 25 using several routes, without grinding (Example 11) and

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with various types of grinding equipment (Examples 12-14).

Materials used:

5 Biomass containing 36.3% of oil to 39.5% of arachidonic acid (ARA).  
High oleic acid sunflower oil.  
Palm olein.  
MCT oil.

10

Example 11 - Preparation by contacting with high oleic acid sunflower oil

Equipment:

15 Stirred glass reactor of 1000 ml with a double jacket, linked to a thermostatted bath.  
Carver press with a 48 x 200 mm filtration cartridge.  
Thermostatted bell-shaped filter MAVAG 300 ml.  
Laboratory deodorizer according to J.HEIDE-JENSEN  
20 (JAOCS; Vol. 40, 223-224; 1963) with a 1000 ml round-bottomed flask.

Procedure:

25 260 g of high oleic acid sunflower oil and 112 g of biomass are introduced into the reactor. The vessel is placed under vacuum and the air is replaced with nitrogen three times for the inerting. The reactor is then stirred at 50°C for 2 h, and then the mixture is recovered in a filtration cartridge. The oil is separated from the biomass by pressing. 260 g of oil and 110 g of cake are recovered.  
The pressed oil is filtered at 50°C, and then it is deodorized at 180°C, 1 mbar for 2.6 h. 240 g of a clear oil having a neutral odour and a light yellow colour  
35 are finally obtained. The ARA content of the oil is determined by gas chromatography (GC) analysis and the level of incorporation of ARA is calculated.

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Example 12 - Preparation by grinding with palm olein in a ball mill

Equipment:

5    DYNOMILL type KDL ball mill with 0.3 l grinding vessel, double jacket linked to a thermostatted bath.  
Carver press with a 48 x 200 mm filtration cartridge.  
Thermostatted bell-shaped filter MAVAG 300 ml.  
Laboratory deodorizer according to J.HEIDE-JENSEN  
10 (JAOCS; Vol. 40, 223-224; 1963) with 1000 ml round-bottomed flask.

Procedure:

130 g of palm olein and 56 g of biomass are introduced  
15 into the mill vessel. The vessel is placed in a vacuum and the air is replaced with nitrogen three times for the inerting. 220 ml of glass beads having a diameter of 2 mm are added and the vessel is heated to 65°C with the aid of a thermostatted bath. The mixture is then  
20 ground for 5 min at a temperature of 65 to 75°C, and then the vessel is emptied. The beads are separated from the mixture by filtration on a grid having a diameter of 1 mm, the mixture is recovered in a filtration cartridge and a sample is collected for  
25 measuring the size of the particles. The oil is separated from the biomass by pressing. The procedure is repeated a second time.

225 g of oil and 75 g of cake are recovered. The  
30 pressed oil is filtered at 50°C, and then it is deodorized at 180°C, 1 mbar for 2.2 h. 210 g of a clear oil having a neutral odour and a light yellow colour are finally obtained. The ARA content of the oil is determined by GC analysis and the level of  
35 incorporation of ARA is calculated.

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Example 13 - Preparation by grinding with MCT oil in a ball mill

The trial described in Example 12 is repeated using MCT  
5 oil as a replacement for palm olein. 235 g of oil and  
65 g of cake are finally recovered.

The pressed oil is filtered at 50°C, and then it is  
10 deodorized at 180°C, 1 mbar for 2.3 h. 220 g of a clear  
oil having a neutral odour and a light yellow colour  
are finally obtained. The ARA content of the oil is  
determined by GC analysis and the level of  
incorporation of ARA is calculated.

15 Example 14 - Preparation by grinding with high oleic acid sunflower oil in a colloid mill

Equipment:

FRYMA MZ 80 colloid mill.

20 PADBERG basket centrifuge.

Thermostatted bell-shaped filter MAVAG 300 ml.

Laboratory deodorizer according to J.HEIDE-JENSEN  
(JAOCS; Vol. 40, 223-224; 1963) with a 1000 ml round-  
bottomed flask.

25

Procedure:

2800 g of high oleic acid sunflower oil and 1200 g of  
biomass are introduced into the mill vessel. The  
grinding is carried out by recirculating the mixture in  
30 the grinder under an inert atmosphere for 10 minutes at  
a temperature of 40 to 70°C. The mixture is recovered  
and a sample is collected for measuring the size of the  
particles. The oil is separated from the biomass with  
the aid of the basket centrifuge. 2400 g of oil and  
35 1400 g of cake are finally recovered.

200 g of centrifuged oil are filtered at 50°C, and then  
it is deodorized at 180°C, 1 millibar for 2 hours.  
190 g of a clear oil having a neutral odour and a light

- 15 -

yellow colour are finally obtained. The ARA content of the oil is determined by GC analysis and the level of incorporation of ARA is calculated. The results are indicated in Table 3 below:

5

Table 3:

Example	Size of the particles (Malvern Mastersizer) micron D (v, 0.9)*	g ARA in 100 g oil	% level of incorporation of ARA
11	3000	3.5	66.1
12	75	5.0	94.3
13	60	5.0	94.3
14	115	5.25	99.0

\*Micron, D (v, 0.9): means that 90% by volume of the  
10 particles have a diameter of less than D.

Example 15: Incorporation of DHA into the high oleic acid sunflower oil

15 Equipment:

FRYMA MZ 80 colloid mill.

PADBERG basket centrifuge.

Thermostatted bell-shaped filter MAVAG 300 ml.

Laboratory deodorizer according to J.HEIDE-JENSEN  
20 (JAOCS; Vol. 40, 223-224; 1963) with a 1000 ml round-bottomed flask.

Procedure:

The procedure of Example 14 is repeated by treating  
25 1200 g of a biomass containing 25% of an oil with a DHA content of 40%. 2500 g of oil with a DHA content of 3.5% are recovered, which oil is deodorized.

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Examples 16-21

16-17. An infant formula for premature babies enriched with ARA is prepared from the oil prepared by the process of Examples 12 or 13 and there are added thereto other oils, for example in the proportions indicated in Table 4 below, proteins, where appropriate hydrolyzed, carbohydrates and where appropriate vitamins and trace elements.

10

Table 4

	Example 16	Example 17
Oil of Example 12	4	-
Oil of Example 13	-	4
Fish oil	1.5	1.5
MCT oil	27	25
Soya bean oil	23	23
Palm olein	44.5	35
High oleic acid sunflower oil	-	11.5
Total	100	100

18-19. An infant formula for full-term unweaned babies enriched with ARA is prepared from the carrier oil prepared by the process of Examples 13 or 14 and there are added thereto other oils, for example in the proportions indicated in Table 5 below, proteins, where appropriate hydrolyzed, carbohydrates and where appropriate vitamins and trace elements.

15

20

Table 5

	Example 18	Example 19
Oil of Example 13	4.5	-
Oil of Example 14	-	7
Fish oil	1.5	1.5
Coconut oil	20	27.5

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	Example 18	Example 19
Soya bean oil	20	20
Palm olein	54	44
Total	100	100

20-21. A follow-on milk for small children enriched with ARA is prepared from the carrier oil prepared by the process of Example 12 or enriched with DHA from the  
 5 carrier oil prepared by the process of Example 15, and there are added thereto other oils in the proportions indicated in Table 6 below, proteins, where appropriate hydrolyzed, carbohydrates and where appropriate vitamins and trace elements.

10

Table 6

	Example 20	Example 21
Oil of Example 12	4	-
Oil of Example 15	-	7
Fish oil	1.5	-
Palm kernel oil	27	-
Coconut oil	-	19
Soya bean oil	23	-
Rapeseed oil	-	30
Palm olein	44.5	44
Total	100	100

Example 22

15

A liquid milk enriched with DHA in an amount of 1% of DHA in the fatty phase is prepared in the following manner:

20 A whole milk containing 3.92% of fat and 8.58% of solids-not-fat and a low-fat milk containing 0.05% of fat and 9% of solids-not-fat are pasteurized separately by treating them at 87°C for 12 s.

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34.69 kg of whole milk and 160.26 kg of low-fat milk, cooled to 15°C are then mixed, and then a premix of 1.08 kg of oil obtained according to Example 15 (high oleic acid sunflower oil, containing 3.5% of DHA),  
5 1.08 kg of soya bean oil and 1 g of vitamin E heated to 50°C is incorporated into this mixture by means of a colloid mill.

Sterilized product:

10 After heating to 80°C in a plate exchanger, the liquid is UHT sterilized at 148°C for 5 s. After cooling at 78°C, it is homogenized in two stages, at 200 bar, and then at 50 bar; it is cooled to 20°C and it is aseptically packaged in carton-type packaging which has  
15 been previously sterilized, the homogenization, cooling and filling stages taking place aseptically.

Pasteurized product:

The liquid is heated at 72°C for 15 s in a plate  
20 exchanger; it is homogenized in two stages at 200 bar, and then at 50 bar; it is cooled to 4°C and it is packaged in carton-type packaging.

Example 23

25 As a nutritional supplement, an oil prepared according to Example 12, 13 or 14 containing ARA or an oil prepared according to Example 15 containing DHA, is encapsulated in an amount of 500 mg of oil in gelatine  
30 capsules.

Claims

1. Stable oil entering into the composition of a food, nutritional, pharmaceutical or cosmetic product intended for human or animal use, containing one or more long-chain polyunsaturated fatty acids obtained from a biomass, in the form of triacylglycerols serving both as transfer medium for the said long-chain polyunsaturated fatty acids from the biomass and as carrier for the said long-chain polyunsaturated fatty acids in the said food, nutritional, pharmaceutical or cosmetic product and into which the long-chain polyunsaturated fatty acids are incorporated such that at least 60% by weight of the long-chain polyunsaturated fatty acids present in the biomass is now in the oil and that less than 10% of the phosphorus present in the biomass is now in the oil.  
10  
15
2. Oil according to Claim 1, in which the long-chain polyunsaturated fatty acids are chosen from arachidonic acid, dihomogammalinolenic acid, eicosapentaenoic acid or docosahexaenoic acid.  
20
3. Oil according to Claim 2, in which the long-chain polyunsaturated fatty acid is arachidonic acid.  
25
4. Oil according to Claim 2, in which the long-chain polyunsaturated fatty acid is docosahexaenoic acid.
- 30 5. Process for preparing an oil according to one of Claims 1 to 4, in which a carrier oil, entering into the composition of a food, nutritional, pharmaceutical or cosmetic product, is brought into contact with a biomass obtained from the culture of a microorganism containing one or more long-chain polyunsaturated fatty acids, so as to transfer the long-chain polyunsaturated fatty acid(s) in the form of triacylglycerols to the said carrier, the oil containing the said fatty acid(s) is separated from the biomass residue by pressing and  
35

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filtration and it is deodorized under gentle conditions.

6. Process according to Claim 5, in which the sizes  
5 of the particles of dry biomass are reduced in order to  
break the walls of the cells of microorganisms and to  
thereby increase the surface area of contact between  
the oil and the biomass, by grinding it before mixing  
it with the carrier oil.

10 7. Process according to Claim 5, in which the biomass  
is treated at high pressure in the presence of the  
carrier oil, and then the oil obtained, containing the  
long-chain polyunsaturated fatty acid, is separated  
15 from the biomass by pressing and filtration.

8. Process according to Claim 5, in which the biomass  
is subjected to grinding in the presence of the carrier  
oil under gentle conditions, at a moderate temperature  
20 under an inert atmosphere, in particular under a  
nitrogen layer.

9. Process according to Claims 5 to 8, in which a  
final filtration is carried out in order to remove the  
25 fine particles of biomass.

10. Foodstuff containing an oil according to one of  
Claims 1 to 4.

30 11. Infant foodstuff containing an oil according to  
one of Claims 1 to 4.

12. Nutritional composition containing an oil  
according to one of Claims 1 to 4.

35 13. Cosmetic composition in dry or emulsion form  
containing an oil according to one of Claims 1 to 4.

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14. Foodstuff containing an oil according to one of  
Claims 1 to 4 intended as animal feed.

15. Foodstuff containing the biomass residue obtained  
5 by the process according to one of Claims 5 to 8  
intended as animal feed.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 02/02333

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>				
IPC 7 C11B1/10 A23C11/04	C11B1/06 A23K1/16	A23D9/00 A61K7/48	C12P7/64	A23L1/30

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 C11B A23D C12P A23L A23C A23K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, FSTA, BIOSIS, CHEM ABS Data, CAB Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 726 321 A (OMEGATECH INC) 14 August 1996 (1996-08-14) page 6, line 27 -page 7, line 6 claims 1,3,8-11 ---	1-3,5,6, 10-12,14
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 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

## \* Special categories of cited documents :

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- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the International search

11 July 2002

Date of mailing of the international search report

24/07/2002

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 02/02333

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